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ARTICLE

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Cecilie Kyrø, Anja Olsen, Rikard Landberg, Guri Skeie, Steffen Loft, Per Åman, Max Leenders, Vincent K. Dik, Peter D. Siersema, Tobias Pischon, Jane Christensen, Kim Overvad, Marie-Christine Boutron-Ruault, Guy Fagherazzi, Vanessa Cottet, Tilman Kühn, Jenny Chang-Claude, Heiner Boeing, Antonia Trichopoulou, Christina Bamia, Dimitrios Trichopoulos, Domenico Palli, Vittorio Krogh, Rosario Tumino, Paolo Vineis, Salvatore Panico, Petra H. Peeters, Elisabete Weiderpass, Toril Bakken, Lene Angell Åsli, Marcial Argüelles, Paula Jakszyn, María-José Sánchez, Pilar Amiano, José María Huerta, Aurelio Barricarte, Ingrid Ljuslinder, Richard Palmqvist, Kay-Tee Khaw, Nick Wareham, Timothy J. Key, Ruth C. Travis, Pietro Ferrari, Heinz Freisling, Mazda Jenab, Marc J. Gunter, Neil Murphy, Eilo Riboli, Anne Tjønneland, H.B(as). Bueno-de-Mesquita

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Correspondence to: Cecilie Kyrø, MSc, PhD, Danish Cancer Society Research Center, Strandboulevarden 49, 2100 Copenhagen Ø, Denmark (e-mail: ceciliek@cancer.dk).

Background Few studies have investigated the association between whole-grain intake and colorectal cancer. Because whole-grain intake estimation might be prone to measurement errors, more objective measures (eg, biomarkers) could assist in investigating such associations.

Methods The association between alkylresorcinols, biomarkers of whole-grain rye and wheat intake, and colorectal cancer incidence were investigated using prediagnostic plasma samples from colorectal cancer case patients and matched control subjects nested within the European Prospective Investigation into Cancer and Nutrition. We included 1372 incident colorectal cancer case patients and 1372 individual matched control subjects and calculated the incidence rate ratios (IRRs) for overall and anatomical subsites of colorectal cancer using conditional logistic regression adjusted for potential confounders. Regional differences (Scandinavia, the Mediterranean, Central Europe) were also explored.

Results High plasma total alkylresorcinol concentration was associated with lower incidence of distal colon cancer; the adjusted incidence rate ratio of distal colon cancer for the highest vs lowest quartile of plasma total alkylresorcinols was 0.48 (95% confidence interval [CI] = 0.28 to 0.83). An inverse association between plasma total alkylresorcinol concentrations and colon cancer was found for Scandinavian participants (IRR per doubling = 0.83; 95% CI = 0.70 to 0.98). However, plasma total alkylresorcinol concentrations were not associated with overall colorectal cancer, proximal colon cancer, or rectal cancer. Plasma alkylresorcinols concentrations were associated with colon and distal colon cancer only in Central Europe and Scandinavia (ie, areas where alkylresorcinol levels were higher).

Conclusions High concentrations of plasma alkylresorcinols were associated with a lower incidence of distal colon cancer but not with overall colorectal cancer, proximal colon cancer, and rectal cancer.

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Colorectal cancer is the third most common type of cancer worldwide (1). Dietary and lifestyle habits have been proposed to account for a large proportion of colorectal cancers (2). Recently, the Continuous Update Project of the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) Expert Report upgraded the evidence for an inverse association between dietary fiber intake and colorectal cancer risk to "convincing" (3). In particular, evidence suggests that intake of cereal fibers is more strongly associated with decreased colorectal cancer risk (4). Dietary fiber, and especially cereal fiber, would be expected to also represent whole-grain intake (5). Few studies have investigated the role of whole grains as such in colorectal cancer prevention because most prospective cohort studies have poor or no information on dietary intake of whole grains (6).

Adequate exposure measurement is one of the greatest challenges in nutritional epidemiology, and most prospective studies use food frequency questionnaires (FFQs) for dietary assessment. However, FFQs and other dietary assessment methods are prone to exposure misclassification, which might lead to attenuation of the diet–disease relationship under study (7). Previous studies on dietary fiber and colorectal cancer have found that methodological differences, especially regarding exposure measurement, might account for null

findings in some studies (8). Whole-grain intake might be even more prone to measurement errors than other food products because consumers may have difficulties in accurately identifying whole-grain products and the actual whole-grain content varies greatly among whole-grain products (5,9). Therefore, using biomarkers of whole-grain intake could be one attractive option to overcome some of these problems. Alkylresorcinols (1,3-dehydroxy-5-alkylbenzene homologs) are phenolic lipids found exclusively in the bran part of wheat, rye, and, to a very minor extent, barley among commonly consumed foods (10). Low or trace content is found in refined products (11). Alkylresorcinols are not affected by food processing (12), are absorbed in the small intestine (13), and can be measured in blood plasma (14). Intervention studies have shown that plasma alkylresorcinol concentrations are highly affected by dietary intake of whole-grain wheat and rye (15). In observational studies, moderate correlations (r = 0.25–0.57) have been found when comparing the plasma level of alkylresorcinols with different dietary intake measures (6,16). Despite the apparent short half-life, the long-term reproducibility (time period of 0.1–4 years) was moderate to good in populations with frequent whole-grain intake (17,18). Five alkylresorcinol homologs are generally analyzed, and in human plasma the ratio of two of these (C17:0/C21:0) is typically less than 0.2 if the consumed whole-grain mostly consists of wheat and is greater if whole-grain rye is also consumed (15,19).

In this study, we examined the association between plasma total alkylresorcinol concentrations as well as the C17:0/C21:0 ratio as an indicator of whole-grain source (wheat or rye) consumed and risk of overall colorectal cancer and colorectal cancer anatomical subsites in a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC).

Methods

Study Population and Data Collection

The EPIC study is a large, multicenter cohort study that includes more than half a million European participants. The cohort consists of 23 centers in Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. Most participants were recruited from the general population. In this study, only one of the two Swedish cohorts participating in EPIC is included (Umeå). At baseline (years 1993–1998), lifestyle questionnaires, FFQs, and anthropometric measurements were collected from the participants (20).

Biological samples, including plasma samples, were collected at baseline from 385 747 of the 519 978 EPIC cohort participants and stored in nitrogen vapor or liquid nitrogen at less than –150 °C for later use, with the exception of the Swedish samples, which were stored in –80 °C freezers (20).

This study was approved by the Ethical Review Board at the International Agency for Research on Cancer (IARC) and the ethical committees of the participating centers. All participants provided informed consent.

Follow-up for Cancer Incidence and Vital Status

Cancer incidence was identified through record linkage with population cancer registries in most centers (20). End of follow-up ranged from December 2002 to June 2005. During follow-up, 2391 cohort members were diagnosed with colorectal cancer, 1550 of whom were randomly selected to be part of this study.

Case Ascertainment and Selection

Colorectal cancer case patients were identified in accordance with the 10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death and the Second Edition of the International Classification of Diseases for Oncology. Proximal colon cancers were located in appendix, cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure (C18:0–C18:5). Distal colon cancer included descending (C18:6) and sigmoid colon cancers (C18:7). Overlapping (C18:8) and unspecified lesions (C18:9) of the colon were grouped among all colon cancers only (C18:0–C18:9). Cancers of the rectosigmoid junction (C19:9) and cancers of the rectum (C20) were grouped as rectal cancer.

Matching

The 1550 cohort participants who were diagnosed with colorectal cancer during the follow-up period were matched 1:1 with control subjects using incidence density sampling from eligible cohort members who were alive and free of cancer at the time of the case patient's diagnosis. Case patients were matched individually to control subjects by sex, study center, age at blood collection (±5 years), date of blood collection (±6 months), time of blood collection (±4 hours), and fasting status (no: <3 hours, in-between: 3–6 hours, yes: >6 hours). Women were further matched by menopausal status, phase of menstrual cycle, and use of oral contraceptives or hormone replacement therapy at time of blood collection. The case–control set was also used for other studies, and for that reason not all matching factors are relevant for this study (such as phase of menstrual cycle).

Laboratory Analyses

Plasma alkylresorcinol homologs (C17:0, C19:0, C21:0, C23:0, C25:0) concentrations were determined by a gas chromatography–mass spectrometry method in which molecular ions were used for quantification in single ion monitoring mode (21). Matched case–control pairs were analyzed in the same batch, and quality control samples were included in each batch. The within- and between-day precisions, expressed as coefficients of variation, were 11% and 22%, respectively. Total plasma concentration (sum of homologs C17:0, C19:0, C21:0, C23:0, and C25:0) was used in the statistical analyses. Alkylresorcinols were successfully analyzed in only 2849 samples of the initial 3100 samples, mostly because of missing samples or insufficient volume rather than laboratory errors.

Exclusions

Of the 2849 available samples, 34 colorectal cancer case patients were excluded because they had noncarcinomas and therefore had different pathology and possibly etiology. Furthermore, 71 participants were excluded because they or their matched case patient or control subject had missing data on covariables. In total, 1372 complete case–control sets (n = 2744 participants in total) were included in the statistical analyses.

Statistical Analyses

Conditional logistic regression stratified by case–control pair was used to estimate odds ratios and 95% confidence intervals (CIs) of

colorectal cancer and anatomical subsites of colorectal cancer in relation to total alkylresorcinol concentration. Because of the incidence density sampling method used, the estimated odds ratios are approximately the same as incidence rate ratios (IRRs) (22). Risk estimates are therefore presented as incidence rate ratios.

In the conditional logistic regression models, plasma total alkylresorcinol concentration was $\log_2$ transformed, meaning that the continuous risk estimates were expressed for doublings of plasma alkylresorcinol concentration (nmol/L). The associations were further expressed as sex-specific quartiles based on the plasma total alkylresorcinol concentration among the control subjects.

Three conditional logistic regression models were constructed. The first was only conditioned on matching factors. The second model was also conditioned on matching factors and further adjusted for potential confounders. A third model was further adjusted for dietary folate intake because it has been questioned whether observed associations between fiber (and thereby possibly whole grains) and the risk of colorectal cancer are confounded by folate intake (23,24). This could potentially be a problem in European observational studies because folic acid fortification is not mandatory and thus not widespread in Europe (23,24). All factors classified as "convincing" or "probable" risk factors of colorectal cancer according to the report by the Continuous Update Project of the WCRF/AICR (3) were also investigated as potential confounders. Furthermore, factors previously identified as being associated with whole-grain intake (eg, intake of fruits, vegetables, and dairy products) were also evaluated (25). Because no other factors than those that are established risk factors for colorectal cancer affected the results (10% rule) (26), only the following were included in the adjusted model: body mass index (kg/m^2, continuous), smoking status (current, former, never), intake of red and processed meat (any intake, yes/no; g/day, continuous), education (none, primary school, technical/professional school, secondary school, longer education including university, not specified/missing), and physical activity according to the Total Physical Activity Index (inactive, moderately inactive, moderately active, active) (27), alcohol intake (abstainer, yes/no; g/day, continuous). The linearity of the associations was evaluated graphically by linear splines with three boundaries placed at quartiles among case patients. No departures from linearity were found.

Competing risk tests were performed to investigate whether colon and rectum cancer and distal and proximal colon cancer could be merged (28). Tests for heterogeneity by region, fasting status, and sex were performed using Wald's test. The regions were defined as follows: Scandinavia (Norway, Sweden, and Denmark), Central Europe (the United Kingdom, the Netherlands, and Germany), and the Mediterranean (France, Italy, Greece, and Spain). Effect modification by body mass index, meat intake, smoking status, and physical activity level were also investigated, and testing deviation from interaction was done by introducing a product term between exposure and potential effect modifiers.

Exclusion of case–control sets in which the case patients were diagnosed within the first year or within the first 2 years after baseline did not change the results (results not shown).

To investigate whether whole-grain wheat or rye were differentially associated with site-specific colorectal cancer, we calculated the ratio of alkylresorcinol homologs C17:0 and C21:0 and included this as a continuous predictor in the conditional logistic regression model. Two hierarchical models were estimated to test the linearity of C17:0/C21:0. More specifically, we fitted a model with the ratio C17:0/C21:0 as a linear term, then a model with the ratio C17:0/C21:0 as an orthogonal polynomial of order three. Model reduction was tested by means of likelihood ratio tests, and no difference was found ($P > .05$). The model was conditioned on matching factors and adjusted for plasma total alkylresorcinol concentration.

Correlations between plasma total alkylresorcinol concentrations (geometric mean) and intake of cereal fiber estimated from FFQs were investigated using Pearson correlations.

All statistical tests were two-sided. A P value of less than .05 was considered statistically significant.

The statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC) and using R (R Foundation for Statistical Computing, Vienna, Austria). In SAS, the univariate and freq procedures were used for the descriptive statistics, and the phreg procedure was used for the conditional logistic regression models.

Results

Characteristics of Study Subjects

Colorectal cancer case patients (n = 1372) and their individual matched control subjects (n = 1372) were evenly distributed on matching factors as part of the study design (eg, sex, age, fasting status). Case patients were less likely to be physically active and had a higher energy intake, a slightly higher alcohol intake, and a higher intake of cereal products (Table 1). Additionally, case patients had a lower intake of breakfast cereals and dietary folate and a slightly lower plasma total alkylresorcinol concentration. The median concentration of plasma total alkylresorcinols was 54 (5–95th percentile = 14–269) nmol/L among the participants from Scandinavia, 53 (5–95th percentile = 12–329) nmol/L among the Central European participants, and 15 (5–95th percentile = 6–91) nmol/L among the Mediterranean participants. Plasma total alkylresorcinols was moderately correlated with cereal fiber intake estimated from FFQs ($r = 0.33$; $P < .001$).

Associations Between Total Alkylresorcinols and Colorectal Cancer and Anatomical Subsites of Colorectal Cancer

No association was found between total alkylresorcinols and incidence of overall colorectal cancer, rectal cancer, colon cancer, and proximal colon cancer (Table 2). For distal colon cancer, however, an inverse association was observed. A doubling in the plasma alkylresorcinol concentration was associated with a 17% lower incidence of distal colon cancer (adjusted IRR = 0.83; 95% CI = 0.73 to 0.95). When comparing the highest quartile with the lowest quartile, a 52% lower incidence of distal colon cancer was found in the highest quartile (adjusted IRR = 0.48; 95% CI = 0.28 to 0.83). Further adjustment for dietary folate slightly attenuated the association, but it remained statistically significant (adjusted IRR, highest vs lowest quartile = 0.53; 95% CI = 0.30 to 0.93). Competing risk tests showed that it was acceptable to merge colon and rectal cancers and distal and proximal colon cancers ($P \geq .33$).

Table 1. Characteristics of 1372 colorectal cancer case patients and their 1372 matched control subjects*

Characteristic	Case patients	Matched control subjects
Men, No.†	714	714
Women, No.†	658	658
Total, No.	1372	1372
Colorectal cancer subtype		
Colorectal cancer	1372	—
Rectal cancer	501	—
Colon cancer	871	—
Proximal colon cancer	367	—
Distal colon cancer	427	—
Other colon cancer	77	—
Age at blood collection, y, median (P5–P95)†	59 (46–70)	59 (46–71)
Fasting status, %†		
Not fasting, <3 h	47	47
In-between, 3–6 h	22	22
Fasting, >6 h	29	29
Unknown	2	2
BMI, kg/m^2, median (P5–P95)	26 (21–34)	26 (21–33)
Smoking status, %		
Never	41	42
Former	34	33
Current smoker	24	24
Unknown	1	1
Highest education level, %		
None	5	5
Primary school completed	36	37
Technical/professional school	24	25
Secondary school	15	13
Longer education, including university	17	18
Unknown	3	2
Physical activity index, %		
Inactive	15	12
Moderately inactive	28	26
Moderately active	40	43
Active	9	11
Unknown	8	8
Energy intake, Kcal/d, median (P5–P95)	2070 (1169–3285)	2025 (1259–3290)
Alcohol abstainers, %	12	12
Alcohol, g/d, median (P5–P95)‡	11 (0–64)	10 (0–60)
Red meat, g/d, median (P5–P95)	47 (5–125)	45 (5–120)
Processed meat, g/d, median (P5–P95)	25 (1–93)	24 (2–88)
Cereal and cereal products, g/d, median (P5–P95)	195 (76–426)	191 (77–423)
Breakfast cereals, g/d, median (P5–P95)	0 (0–73)	0 (0–107)
Bread and crisp bread, g/d, median (P5–P95)	119 (34–282)	120 (32–291)
Bread, nonwhite, g/d, median (P5–P95)	63 (0–210)	63 (0–213)
Dietary fiber, g/d, median (P5–P95)	22 (12–36)	23 (12–37)
Cereal fiber, g/d, median (P5–P95)	8 (3–19)	8 (3–19)
Folate, µg/d, median (P5–P95)	285 (161–495)	291 (162–494)
Alkylresorcinols, nmol/L, median (P5–P95)		
Total, median (P5–P95)	38 (6–239)	39 (8–271)
C17, median (P5–P95)	3 (0–18)	3 (0–19)
C19, median (P5–P95)	8 (1–64)	9 (1–74)
C21, median (P5–P95)	13 (3–97)	13 (3–102)
C23, median (P5–P95)	6 (1–33)	6 (1–36)
C25, median (P5–P95)	6 (1–40)	6 (1–44)

* BMI = body mass index; P5 = 5th percentile; P95 = 95th percentile.

† Matching factor.

‡ Among users only.

Heterogeneity: Region, Fasting Status, and Sex

Because alkylresorcinols are expected to be useful as biomarkers of whole-grain intake especially in populations in which whole-grain wheat and rye are a staple part of the diet (14), heterogeneity by region in the association between plasma total alkylresorcinols and colorectal cancer was also investigated (Figure 1). When investigating the association between plasma total alkylresorcinols and colorectal cancer and anatomical subsites by region,

Table 2. Incidence rate ratios of the association between plasma total alkylresorcinol concentrations and cancers of the colorectum and anatomical subsites for 1372 colorectal cancer case patients and their 1372 matched control subjects in a nested case–control study within the European Prospective Investigation into Cancer and Nutrition, sex-specific quartiles (control subjects) and according to doublings in concentration*

	Quartile of plasma concentrations (plasma total alkylrecorcinol concentration in nmol/L by sex)				Continuous
Cancer site	**Q1 (M: 0 to ≤21 F: 0 to ≤16), IRR (95% CI)**	**Q2 (M:21 to ≤42 F: 16 to ≤35), IRR (95% CI)**	**Q3 (M: 42 to ≤99 F: 35 to ≤84), IRR (95% CI)**	**Q4 (M: >99 F: >84), IRR (95% CI)**	**Per doubling, IRR (95% CI)**
Colorectum					
No. case patients/control subjects	351/347	341/341	373/342	307/342	1372/1372
Matching factors†	1.00 (referent)	0.98 (0.78 to 1.24)	1.06 (0.83 to 1.36)	0.85 (0.64 to 1.11)	0.95 (0.89 to 1.01)
Multivariable adjusted‡	1.00 (referent)	0.99 (0.78 to 1.25)	1.09 (0.84 to 1.41)	0.86 (0.65 to 1.14)	0.95 (0.89 to 1.02)
Multivariable adjusted§	1.00 (referent)	1.00 (0.78 to 1.26)	1.10 (0.85 to 1.42)	0.87 (0.66 to 1.16)	0.96 (0.89 to 1.02)
Rectum					
No. case patients/control subjects	129/122	109/119	137/129	126/131	501/501
Matching factors†	1.00 (referent)	0.85 (0.57 to 1.26)	0.97 (0.64 to 1.49)	0.86 (0.55 to 1.36)	0.95 (0.85 to 1.05)
Multivariable adjusted‡	1.00 (referent)	0.78 (0.52 to 1.19)	0.92 (0.59 to 1.42)	0.83 (0.52 to 1.32)	0.94 (0.84 to 1.04)
Multivariable adjusted§	1.00 (referent)	0.78 (0.52 to 1.19)	0.92 (0.59 to 1.42)	0.83 (0.52 to 1.32)	0.94 (0.84 to 1.04)
Colon					
No. case patients/control subjects	222/225	232/222	236/213	181/211	871/871
Matching factors†	1.00 (referent)	1.06 (0.80 to 1.42)	1.11 (0.81 to 1.51)	0.82 (0.58 to 1.16)	0.95 (0.87 to 1.03)
Multivariable adjusted‡	1.00 (referent)	1.08 (0.81 to 1.46)	1.13 (0.82 to 1.56)	0.83 (0.58 to 1.18)	0.95 (0.87 to 1.03)
Multivariable adjusted§	1.00 (referent)	1.10 (0.82 to 1.48)	1.15 (0.84 to 1.59)	0.85 (0.60 to 1.22)	0.96 (0.88 to 1.04)
Proximal colon					
No. case patients/control subjects	92/93	92/95	99/94	84/85	367/367
Matching factors†	1.00 (referent)	0.99 (0.64 to 1.53)	1.07 (0.68 to 1.67)	1.01 (0.61 to 1.67)	1.01 (0.89 to 1.13)
Multivariable adjusted‡	1.00 (referent)	1.08 (0.68 to 1.71)	1.18 (0.73 to 1.90)	1.09 (0.64 to 1.85)	1.02 (0.90 to 1.15)
Multivariable adjusted§	1.00 (referent)	1.08 (0.68 to 1.72)	1.18 (0.73 to 1.90)	1.09 (0.64 to 1.85)	1.02 (0.90 to 1.15)
Distal colon					
No. case patients/control subjects	109/92	120/106	120/104	78/114	427/427
Matching factors†	1.00 (referent)	1.03 (0.68 to 1.56)	0.96 (0.60 to 1.53)	0.49 (0.29 to 0.82)	0.84 (0.74 to 0.95)
Multivariable adjusted‡	1.00 (referent)	1.05 (0.68 to 1.62)	0.93 (0.57 to 1.51)	0.48 (0.28 to 0.83)	0.83 (0.73 to 0.95)
Multivariable adjusted§	1.00 (referent)	1.09 (0.70 to 1.70)	0.99 (0.60 to 1.63)	0.53 (0.30 to 0.93)	0.85 (0.74 to 0.97)

* CI = confidence interval; F = female; M = male; IRR = incidence rate ratio; Q = quartile.

† Model conditioned on matching factors: age, sex, study center, time of day of blood collection, and fasting status. Women were also matched on menopausal status, phase of menstrual cycle, and use of hormone replacement therapy or oral contraceptives.

‡ Model conditioned on matching factors plus further adjustments for body mass index, intake of red and processed meat, physical activity, smoking status, education, and alcohol intake.

§ Model same as ‡ but further adjusted for folate intake.

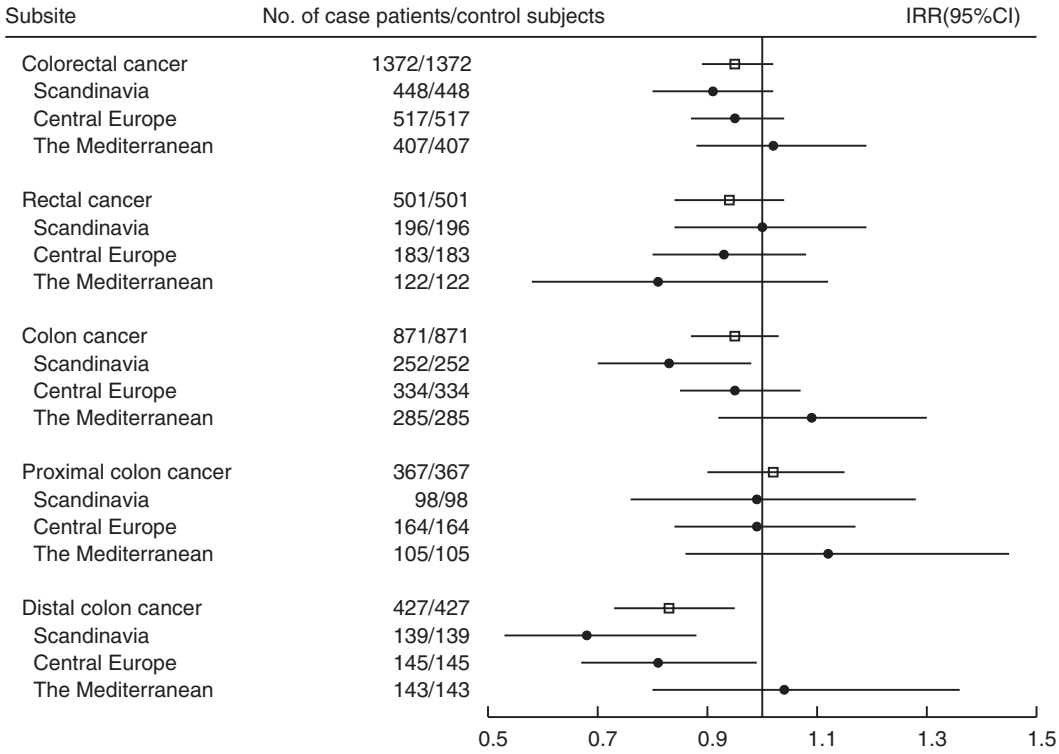

Figure 1. Forest plot of regional and pooled incidence rate ratios (IRRs) and 95% confidence intervals (CIs) for the association between doubling in plasma concentration of alkylresorcinols with incidence of overall colorectal cancer and anatomical subsites, including rectal cancer, colon cancer, proximal colon cancer, and distal colon cancer. Incidence rate ratios were conditioned on matching factors (age, sex, study center, time of day of blood collection, and fasting status; for women, also menopausal status, phase of menstrual cycle, and use of hormone replacement therapy or oral contraceptives) and further adjusted for body mass index, intake of red and processed meat, physical activity, smoking status, education, and alcohol intake. The **squares** indicate the point estimates of incidence rate ratio for all regions pooled, and the **circles** indicate the point estimates for incidence rate ratios by region (Scandinavia, Central Europe, and the Mediterranean). The **lines** indicate the 95% confidence intervals.

the inverse association for distal colon cancer was present for Scandinavia and Central Europe (Central Europe: adjusted IRR, per doubling = 0.81, 95% CI = 0.67 to 0.99; Scandinavia: adjusted IRR, per doubling = 0.68, 95% CI = 0.53 to 0.88) but not for the Mediterranean (adjusted IRR, per doubling = 1.04, 95% CI = 0.80 to 1.36). Furthermore, a statistically significant association was also found for overall colon cancer for Scandinavia (adjusted IRR, per doubling = 0.83; 95% CI = 0.70 to 0.98).

When testing for heterogeneity, no statistically significant difference was found between the three regions for either overall colorectal cancer or distal colon cancer (results not shown). When testing for heterogeneity between Scandinavia and Central Europe together vs the Mediterranean, a difference was found for distal cancer (P = .04) but not for overall colorectal cancer (P = .25). No heterogeneity was found between categories of fasting status or sex; however, associations seemed slightly stronger for men (Women: IRR, conditioned on matching factors, distal colon = 0.88, 95% CI = 0.74 to 1.04; Men: IRR, conditioned on matching factors, distal colon = 0.79, 95% CI = 0.65 to 0.95).

Whole-Grain Wheat- or Rye-Dominated Diet

The ratio between the alkylresorcinol homologs C17:0 and C21:0 (C17:0/C21:0) indicates whether the diet is dominated by whole-grain wheat or rye (Figure 2). The association between the ratio C17:0/C21:0 and distal colon cancer adjusted for total alkylresorcinol concentration indicated no signs of a stronger inverse association for either whole-grain wheat or rye.

Effect modification

No signs of effect modification by body mass index, smoking status, physical activity, or intake of red and processed meat were found (Supplementary Table 1, available online).

Discussion

In this prospective study, which included participants from 10 European countries, plasma total alkylresorcinol concentrations were inversely associated with risk of distal colon cancer. In the Scandinavian part of the cohort, plasma total alkylresorcinol concentrations were also inversely associated with colon cancer. No associations with other anatomical subsites of colorectal cancer (rectum and proximal colon) were seen. No difference in the association with distal colon cancer was found depending on the ratio of alkylresorcinol homologs C17:0 and C21:0, indicating that there were no differences depending on whether whole-grain wheat or whole-grain rye was chiefly consumed.

Heterogeneity by geographical region was observed when comparing associations for Scandinavia and Central Europe with the Mediterranean. The inverse association with distal colon cancer was only observed for Scandinavia and Central Europe, which

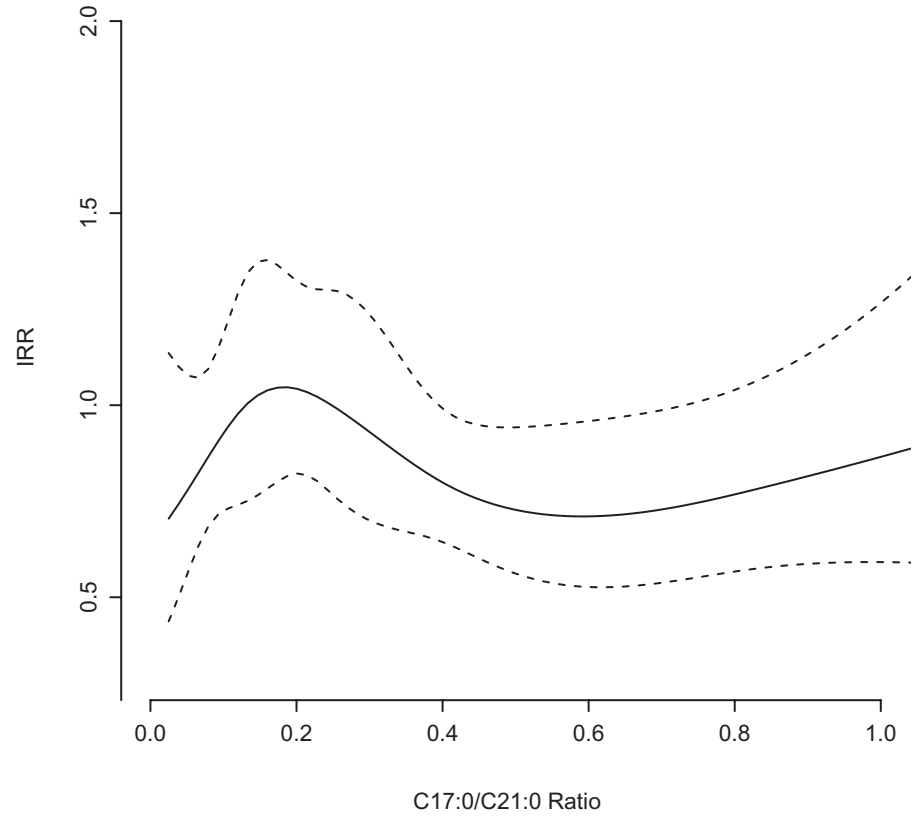

Figure 2. Association between the ratio of the two alkylresorcinol homologs C17:0 and C21:0 and distal colon cancer. Model adjusted for plasma total alkylresorcinol concentration and conditioned on matching factors. In human plasma alkylresorcinol, the C17:0/C21:0 ratio is typically 0.1 to 0.2 when the diet is dominated by whole-grain wheat and 0.6 to 0.8 when the diet is dominated by whole-grain rye. IRR = incidence rate ratio.

might be a consequence of stable and high whole-grain intakes in these regions, as well as a wider intake range.

Our findings are suggestive of a protective effect of whole-grain intake on colon cancer development, especially of distal colon cancer. The association is especially strong for the fourth quartile. Therefore it can be questioned whether the association is linear. However, the association is also statistically significant when assessed by doubling in concentrations, and linear splines also indicated that the association is linear (data not shown).

The association between whole-grain intake and colorectal cancer has been investigated in some cohort studies, but few studies are available, and the results have been inconsistent (29–36). A recent published meta-analysis reported an inverse association between whole-grain intake and incidence of colorectal cancer (4). A number of mechanisms have been suggested as being responsible for this inverse association, some of which include improved bowel emptying, preventive effects of the short-chain fatty acid butyrate produced by fermentation in the colon, possible antioxidative effects, and dilution and entrapment of carcinogenic substances (37).

The association between plasma alkylresorcinol concentrations and risk of colorectal cancer was strongest for cancers of the distal part of the colon. Accumulating evidence suggests that the etiologies of distal and proximal cancer are likely to be different (38,39). The most recent study on intake of dietary fiber and incidence risk of colorectal cancer in the entire EPIC cohort also found a statistically significant inverse association with distal colon cancer risk only and no association with risk of proximal colon cancer in the uncalibrated analysis (40). When associating cereal fiber intake with colorectal cancer incidence in our study population, the same pattern of an association with distal colon cancer was found. Whole-grain cereals are rich in lignified and resistant fibers present in the bran part of the grains, which are not fermented in the proximal colon but reach the distal colon (41). Because the proximal colon and the distal colon have different embryologic origin and there are differences in the mucosa (39,42), it seems plausible that diet might have different effect on the proximal and the distal colon.

The study has several strengths. First, plasma concentrations of alkylresorcinols are an independent, novel, and valid biomarker of whole-grain intake (14), with modest to good long-term reproducibility in cohorts with frequent and stable intakes (17,18). Moreover, the prospective design with prediagnostic blood samples and the low risk of selection bias because the case–control study is nested within a cohort are considerable strengths. Furthermore, information on many potential confounders was available, and a large number of case patients for whom detailed information on tumor morphology, behavior, and location was available are included.

Our study is not without limitations. Plasma levels of alkylresorcinols are influenced by between-subject differences in metabolism, which would lead to an attenuation of the association under investigation (14). Because of the apparent short half-life of approximately 5 hours, alkylresorcinol concentrations fluctuate substantially over time unless frequent intake is evident, and this will further contribute to regression dilution bias. This problem

is further accentuated when using nonfasting samples (43). In this study, both fasting and nonfasting samples were used, and the case patients were matched to control subjects by fasting status; however, similar associations were observed when fasting and nonfasting participants were analyzed separately. A statistically significant inverse association was found for distal colon cancer and for overall colon cancer in Scandinavia. Alkylresorcinols are biomarkers only of whole-grain wheat and rye intake, meaning that dietary intakes of other whole-grain cereals such as oats cannot be assessed using this biomarker. The within-batch precision was acceptable and the between-batch precision was somewhat higher than found previously for this method (18,43). This is probably because three individuals analyzed the data in a large number of batches over a long time period. The practical implication is probably small because case–control pairs were analyzed within the same batch and because of overall high between-subject variation.

In summary we found that high plasma total alkylresorcinol concentrations, reflecting high whole-grain wheat and rye intake, were not associated with a lower incidence of overall colorectal cancer, proximal colon cancer, or rectal cancer. However, a statistical inverse association was found with distal colon cancer and furthermore with overall colon cancer for the Scandinavian participants. The association with distal colon cancer was only observed among participants from Central Europe and Scandinavia (ie, in populations in which whole-grain wheat and rye are consumed regularly).

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127(12):2893–2917.
2. World Cancer Research Fund/American Institute for Cancer Research. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*. Washington, DC: AICR; 2007.
3. World Cancer Research Fund/American Institute for Cancer Research. *Continuous Update Project Interim Report Summary. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer, 2011*. http://www.wcrf.org/cancer_research/cup/key_findings/colorectal_cancer.php. Accessed February 1, 2013.
4. Aune D, Chan DS, Lau R, et al. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ*. 2011;343:d6617.
5. Menzel C, Kamal-Eldin A, Marklund M, Andersson A, Aman P, Landberg R. Alkylresorcinols in Swedish cereal food products. *J Food Comp Anal*. 2012;28(2):119–125.
6. Ross AB, Pineau N, Kochhar S, Bourgeois A, Beaumont M, Decarli B. Validation of a FFQ for estimating whole-grain cereal food intake. *Br J Nutr*. 2009;102(11):1547–1551.
7. Illner AK, Freisling H, Boeing H, Huybrechts I, Crispim SP, Slimani N. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. *Int J Epidemiol*. 2012;41(4):1187–1203.
8. Dahm CC, Keogh RH, Spencer EA, et al. Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. *J Natl Cancer Inst*. 2010;102(9):614–626.
9. van Dam RM, Hu FB. Are alkylresorcinols accurate biomarkers for whole grain intake? *Am J Clin Nutr*. 2008;87(4):797–798.
10. Ross AB, Shepherd MJ, Schupphaus M, et al. Alkylresorcinols in cereals and cereal products. *J Agric Food Chem*. 2003;51(14):4111–4118.
11. Ross AB, Kochhar S. Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC-Coularray-based electrochemical detection. *J Agric Food Chem*. 2009;57(12):5187–5193.
12. Landberg R, Kamal-Eldin A, Andersson R, Aman P. Alkylresorcinol content and homologue composition in durum wheat (*Triticum durum*) kernels and pasta products. *J Agric Food Chem*. 2006;54(8):3012–3014.
13. Ross AB, Kamal-Eldin A, Lundin EA, Zhang JX, Hallmans G, Aman P. Cereal alkylresorcinols are absorbed by humans. *J Nutr*. 2003;133(7):2222–2224.
14. Ross AB. Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake. *J Nutr Metab*. 2012;2012:462967.
15. Landberg R, Kamal-Eldin A, Andersson SO, et al. Reproducibility of plasma alkylresorcinols during a 6-week rye intervention study in men with prostate cancer. *J Nutr* 2009;139(5):975–980.
16. Landberg R, Kamal-Eldin A, Aman P, et al. Determinants of plasma alkylresorcinol concentration in Danish post-menopausal women. *Eur J Clin Nutr*. 2011;65(1):94–101.
17. Montonen J, Landberg R, Kamal-Eldin A, et al. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. *Eur J Clin Nutr*. 2010;64(7):698–703.
18. Landberg R, Aman P, Hallmans G, Johansson I. Long-term reproducibility of plasma alkylresorcinols as biomarkers of whole-grain wheat and rye intake within Northern Sweden Health and Disease Study Cohort. *Eur J Clin Nutr*. 2013;67(3):259–263.
19. Kristensen M, Toubro S, Jensen MG, et al. Whole grain compared with refined wheat decreases the percentage of body fat following a 12-week, energy-restricted dietary intervention in postmenopausal women. *J Nutr*. 2012;142(4):710–716.
20. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 2002;5(6B):1113–1124.
21. Landberg R, Aman P, Kamal-Eldin A. A rapid gas chromatography-mass spectrometry method for quantification of alkylresorcinols in human plasma. *Anal Biochem*. 2009;385(1):7–12.
22. Rothman KJ. (ed.) Types of epidemiologic study. *Epidemiology, An Introduction*. New York: Oxford University Press; 2002:57–93.
23. Bingham S. The fibre-folate debate in colo-rectal cancer. *Proc Nutr Soc*. 2006;65(1):19–23.
24. Bingham SA, Norat T, Moskal A, et al. Is the association with fiber from foods in colorectal cancer confounded by folate intake? *Cancer Epidemiol Biomarkers Prev*. 2005;14(6):1552–1556.
25. Kyro C, Skeie G, Dragsted LO, et al. Intake of whole grains in Scandinavia is associated with healthy lifestyle, socio-economic and dietary factors. *Public Health Nutr*. 2011;14(10):1787–1795.
26. Bliss R, Weinberg J, Webster T, Vieira V. Determining the probability distribution and evaluating sensitivity and false positive rate of a confounder detection method applied to logistic regression. *J Biom Biostat*. 2012;3(4):142.
27. The InterAct Consortium. Validity of a short questionnaire to assess physical activity in 10 European countries. *Eur J Epidemiol*. 2012;27(1):15–25.
28. Andersen PK, Borgan O, Keiding N. Regression models. In: Andersen PK, ed. *Statistical Models Based on Counting Processes*. New York: Springer-Verlag; 1993:495–496.
29. Pietinen P, Malila N, Virtanen M, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. *Cancer Causes Control*. 1999;10(5):387–396.
30. Terry P, Giovannucci E, Michels KB, et al. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *J Natl Cancer Inst*. 2001;93(7):525–533.
31. McCullough ML, Robertson AS, Chao A, et al. A prospective study of whole grains, fruits, vegetables and colon cancer risk. *Cancer Causes Control*. 2003;14(10):959–970.
32. Larsson SC, Giovannucci E, Bergkvist L, Wolk A. Whole grain consumption and risk of colorectal cancer: a population-based cohort of 60,000 women. *Br J Cancer*. 2005;92(9):1803–1807.
33. Schatzkin A, Mouw T, Park Y, et al. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. *Am J Clin Nutr*. 2007;85(5):1353–1360.
34. Egeberg R, Olsen A, Loft S, et al. Intake of wholegrain products and risk of colorectal cancers in the Diet, Cancer and Health cohort study. *Br J Cancer*. 2010;103(5):730–734.
35. Wu K, Hu FB, Fuchs C, Rimm EB, Willett WC, Giovannucci E. Dietary patterns and risk of colon cancer and adenoma in a cohort of men (United States). *Cancer Causes Control*. 2004;15(9):853–862.
36. Fung TT, Hu FB, Wu K, Chiuve SE, Fuchs CS, Giovannucci E. The Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets and colorectal cancer. *Am J Clin Nutr*. 2010;92(6):1429–1435.

37. Slavin J. Why whole grains are protective: biological mechanisms. *Proc Nutr Soc*. 2003;62(1):129–134.
38. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. *Ann Intern Med*. 1990;113(10):779–788.
39. Iacopetta B. Are there two sides to colorectal cancer? *Int J Cancer*. 2002;101(5):403–408.
40. Murphy N, Norat T, Ferrari P, et al. Dietary fibre intake and risks of cancers of the colon and rectum in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLoS One*. 2012;7(6):e39361.
41. Harris PJ, Ferguson LR. Dietary fibre: its composition and role in protection against colorectal cancer. *Mutat Res*. 1993;290(1):97–110.
42. Li FY, Lai MD. Colorectal cancer, one entity or three. *J Zhejiang Univ Sci B*. 2009;10(3):219–229.
43. Andersson A, Marklund M, Diana M, Landberg R. Plasma alkylresorcinol concentrations correlate with whole grain wheat and rye intake and show moderate reproducibility over a 2- to 3-month period in free-living Swedish adults. *J Nutr*. 2011;141(9):1712–1718.

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Affiliations of authors: Danish Cancer Society Research Center, Copenhagen, Denmark (CK, AO, JC, AT); Department of Food Science, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden (RL, PÅ); Department of Community Medicine, University of Tromsø, Tromsø, Norway (GS, EW, TB, LAÅ); Department of Public Health, Section of Environmental Health, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark (SL); Department of Gastroenterology and Hepatology (ML, VKD, PDS, BB) and Department of Epidemiology, Julius Center for Health Sciences and Primary Care (PHP), University Medical Center Utrecht, Utrecht, The Netherlands; Molecular Epidemiology Group, Max Delbrueck Center for Molecular Medicine Berlin-Buch, Berlin, Germany (TP); Department of Public Health, Section of Epidemiology, Aarhus University, Aarhus, Denmark (KO); Inserm, Centre for Research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health team, F-94805, Villejuif, France (M-CB-R, GF, VC); Univ Paris Sud, UMRS 1018, F-94805, Villejuif, France (M-CB-R, GF, VC); IGR, F-94805, Villejuif, France (M-CB-R, GF, VC); German Cancer Research Center, DKFZ, Division of Cancer Epidemiology, Heidelberg, Germany (TK, JC-C); Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany (HB); Hellenic Health Foundation, Athens, Greece (ATR, CB, DT); WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece (ATR, CB); Department of Epidemiology, Harvard School of Public Health, Boston, MA (DT); Bureau of Epidemiologic Research, Academy of Athens, Athens, Greece (DT); Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute–ISPO, Florence, Italy (DP); Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy (VK); Cancer Registry and Histopathology Unit, "Civile–M.P.Arezzo" Hospital, ASP Ragusa, Italy (RT); MRC/HPA Centre for Environment and Health (PV) and Department of Epidemiology and Biostatistics (MJG, NM, ER, BB), School of Public Health, Imperial College London, London, UK; Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy (SP); Department of Research, Cancer Registry of Norway, Oslo, Norway (EW); Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden (EW); Samfundet Folkhälsan, Helsinki, Finland (EW); Public Health Directorate, Asturias, Spain (MA); Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Barcelona, Spain (PJ); Andalusian School of Public Health, Granada, Spain (MJS); CIBER de Epidemiología y Salud Pública, Madrid, Spain (MJS, JMH, AB); Public Health Division of Guipuzkoa, Basque Regional Health Department, San Sebastian (PA); Department of Epidemiology, Murcia Regional Health Council, Murcia, Spain (JMH); Navarre Public Health Institute, Pamplona, Spain (AB); Department of Radiation Sciences, Oncology (IL) and Department of Medical Biosciences, Pathology (RP), Umeå University, Umeå, Sweden; Department of Public Health and Primary Care (K-TK, NW) and Medical Research Council Epidemiology Unit (NW), University of Cambridge, Cambridge, UK; Cancer Epidemiology Unit, University of Oxford, Oxford, UK (TK, RCT); International Agency for Research on Cancer, Lyon, France (PF, HF, MJ); National Institute for Public Health and the Environment, Bilthoven, The Netherlands (BB).